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SEXUAL CYCLES AND MATURITY OF THE TURTLE, *CHRYSEMYS PICTA*

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The sexual cycles have been described for male *Sternotherus odoratus* (Risley, 1938), and both sexes of *Terrapene carolina* (Atland, 1951) and *Terrapene ornata* (Legler, 1960). Sexual cycles of the painted turtle, *Chrysemys picta* have been previously described for Nova Scotia females by Powell (1967) and for both sexes of Michigan *C. picta* by Gibbons (1968). A similar study was conducted on Pennsylvania *C. picta* which revealed certain differences in the spermatogenetic and oogenetic cycles and the attainment of sexual maturity as reported by Powell (1967) and Gibbons (1968). The results of this study are presented here.

Methods

This study was conducted at the White Oak Bird Sanctuary, 3 miles north of Manheim, Lancaster County, Pennsylvania. Turtles were captured by hand, with a dip net, or in conventional hoop-net traps. Routine measurements of each turtle included the maximum plastron length, the straight line carapace length, the total shell width at the bridge, and the total shell height at the bridge. The third-claw length of both the fore and hind foot, the preanal, and the postanal tail lengths were also measured to determine the characters of sexual dimorphism (Table I).

Eighty adult specimens of *C. picta* (50 females, 30 males) were dissected and the reproductive tracts were removed to determine the male and female annual sexual cycles.

Fresh ovaries were examined from 30 females having plastron lengths ranging from 106.3 to 136.8 mm, dissected during the periods from 5 August to 31 October, 1966, and from 11 March to 30 July, 1967. Previous examinations (August, 1965) of reproductive tracts of 20 other females had indicated that specimens of 110 mm plastron length were sexually mature. All ovaries were weighed before preservation. Follicles, *corpora lutea,* and *corpora albicantia* were counted and measured after preservation. Follicles less than one millimeter in diameter were not counted. Follicles and oviducal eggs were recorded separate for the right and

left sides. *Corpora lutea* and *albicantia* were studied under a binocular dissecting microscope. No histological studies were made of the female reproductive tract.

Fresh testes were examined from 30 males having plastron lengths ranging from 87.8 to 112.9 mm, dissected during the periods from 23 August to 26 September, 1966 and 5 March to 13 August, 1967. All testes were weighed, and the greater diameter was measured before preservation. The testes were fixed in 10 per cent neutral formalin and after two weeks transferred to 80 per cent ethyl alcohol for storage. Later the testes were embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

All measurements in the field and laboratory were made to the nearest tenth of a millimeter with dial calipers. A triple-beam balance was used to determine all weights.

TABLE 1

A summary of sexual dimorphism in Chrysemys picta

Character	Range	Male mean	S.D.	Range	Female mean	S.D.
Plastron length	70.9–119.2	99.6	10.1	80.8–142.1	120.9	14.2
Shell width	67.0–99.5	81.0	6.6	78.5–114.3	94.4	8.6
Shell height	28.7–49.7	38.2	3.5	31.9–59.5	48.5	6.3
Third forefoot claw	5.1–13.0	10.8	1.4	3.7–9.2	6.6	0.7
Forefoot claw/hind foot claw	2.1–3.2	2.7	0.4	0.9–1.6	1.3	0.3
Preanal tail length	5.7–20.0	13.1	3.2	0.5–14.8	6.8	2.6
Postanal tail length	16.0–33.7	27.3	2.9	21.7–41.0	31.0	4.5
Total tail length	28.9–51.8	40.1	3.6	26.2–45.7	36.9	3.3
Tail length/plastron length	0.38–0.48	0.42	0.03	0.25–0.31	0.28	0.02

RESULTS

Female sexual cycle and maturity

Ovaries weighed the most during April and May (Fig. 1). This corresponds to the mating season and many large ovocytes were found on the ovaries at this time. The ovaries weighed the least during July, August, and September, or after ovulation and nesting has occurred. No correlation was found between plastron length and ovarian weight, as smaller females often had heavier ovaries on the same date as larger females. For example, a female with a plastron length of 107.6 mm sacrificed on 23 August, 1966, had an ovarian weight of 12.6 grams while another female, 136.8 mm in length, sacrificed the same day, had an ovarian weight of only 9.4 grams. Enlarged ovocytes (15 to 25 mm) were present throughout the study periods, but were most numerous during April and May and least numerous during July and August. Cagle (1954) reported that female specimens of *C. picta* collected during May, June, and early July and containing oviducal eggs usually had large ovocytes still present. He assumed that the ovocytes represented second and third broods that would be deposited that same season. Although careful searches were made from April through September, no evidence was found at White Oak to support Cagle. Fresh nests and nesting

females were only found during June. Oviducal eggs were only found during late May, June, and early July. The oviducal eggs examined were pinkish white and slightly translucent, but became white shortly after their removal. They ranged in length from 23.1 to 33.0 mm (mean 28.4) in diameter from 15.4 to 20.1 mm (mean 17.5) and in weight from 5.3 to 6.8 g (mean 5.9).

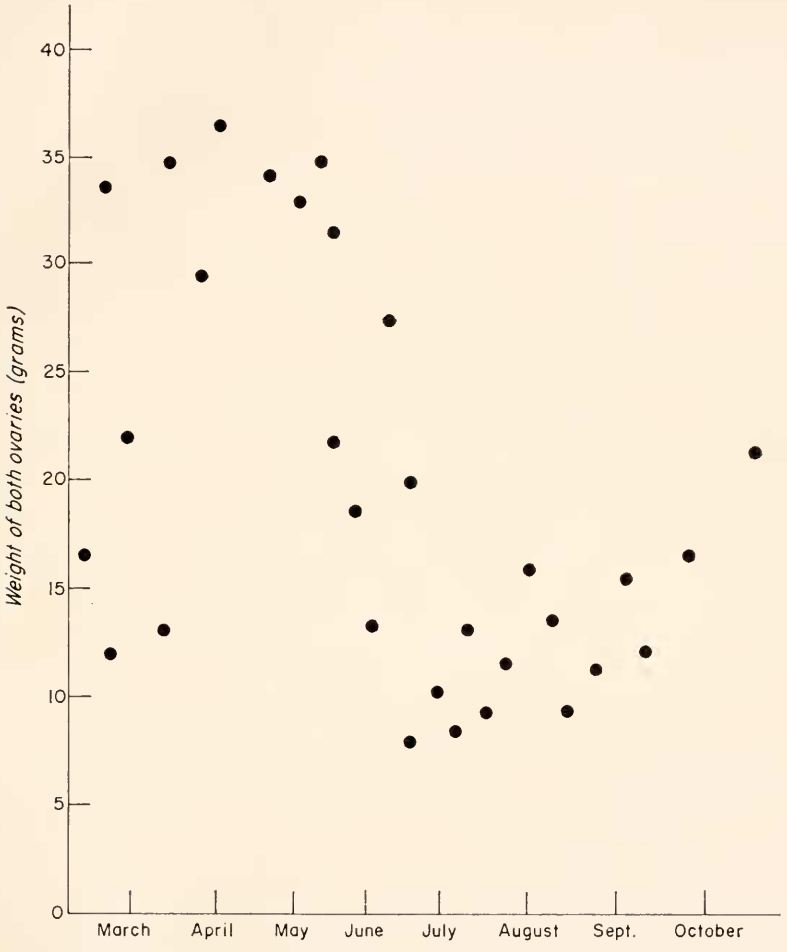

FIGURE 1. Seasonal fluctuations in ovarian weight in thirty specimens of *Chrysemys picta*.

The follicles found on the ovaries could be grouped by diameter as: large (greater than 15 mm), medium (11 to 15 mm), and small (6 to 10 mm) after the system proposed by Legler (1960). Those smaller than 6 mm were not considered. Mature follicles had diameters of 20 to 25 mm. Both Powell (1967) and Gibbons (1968) reported 18 mm as the maximum size of the follicles they measured. (Cagle (1944) considered female specimens of *Chrysemys scripta* to be mature if they contained follicles of 15 mm or greater. All female specimens of

C. picta with the largest follicle 10 mm or more in diameter had two or more additional groups of follicles present on the ovaries (6 to 9 mm in diameter and less than 5 mm). Apparently, these groups represent follicles which would mature and be ovulated during successive mating seasons. None were found to reach mature size before October, and therefore, second nestings as proposed by Cagle (1954), Powell (1967), and Gibbons (1968) would not occur in southeastern Pennsylvania.

The ovarian cycle begins in July and August after ovulation and nesting have occurred. Many small follicles form on the germinal ridges of the ovaries. The ovocytes within the follicles increase in size and by late September and October there is little difference in size between these and the June size of the ovocytes that were not ovulated (about 15 mm). Those not ovulated in June grow and reach

TABLE II

Ovarian activity in ten specimens of Chrysemys picta

Left ovary		Right ovary	
Corpora lutea	Follicles (diameter 15+ mm)	*Corpora lutea*	Follicles (diameter 15+ mm)
4	2	0	4
3	1	1	3
2	5	4	0
2	2	3	1
3	1	1	2
2	3	4	2
5	1	0	3
1	2	5	0
4	2	1	3
4	2	2	3

mature size by early October (about 20 mm). This differs from Gibbons' (1968) and Powell's (1967) findings of little change in size during the summer. There is little change in size during the winter. Atland (1951) suggested that *Terrapene carolina* follicles grow to nearly mature size in the season preceding ovulation and remain quiescent over winter. This appears also to be the case in *C. picta*. Atland also thought that some of the enlarged follicles were absorbed during hibernation. The follicles of White Oak *C. picta* were of mature size in March and remained that way until the ovulation period (May and June). This differs from what Gibbons (1968) found in his Michigan *C. picta*. He reported that specimens taken in March contained yolked follicles 15 to 16 mm in diameter. These remained this size until May when they grew to mature size (about 18 mm).

Ovarian activity alternates in *C. picta*. Counts of *corpora lutea* showed one ovary more active than the other in a given season, and higher counts of enlarged follicles (15 mm in diameter or larger) indicated that the opposite ovary would be more active in the following season (Table II). Legler (1960) reported this same condition in *Terrapene ornata*. The *corpora lutea* are cuplike in shape and 7.5 to 9.0 mm in diameter. The number of oviducal eggs always equalled the

number of *corpora lutea*. Involution of the *corpora lutea* occurs very rapidly and after a month they are barely visible on the ovarian surface and can be referred to as *corpora albicantia*. The *corpora albicantia* disappear when a new clutch of eggs is ovulated.

Extrauterine migration of ova has been reported in *C. picta*, *C. scripta*, *T. ornata*, and *Emydoidea blandingi*, by Legler (1958) and in *Sternotherus odoratus* by Tinkle (1959). An examination of the female painted turtles from which oviducal eggs were removed revealed no evidence of ovular migration. All had equal numbers of *corpora lutea* and oviducal eggs on the same side. Legler (1960) thought such migration may serve to redistribute eggs to the oviducts when the ovaries are functioning at unequal rates.

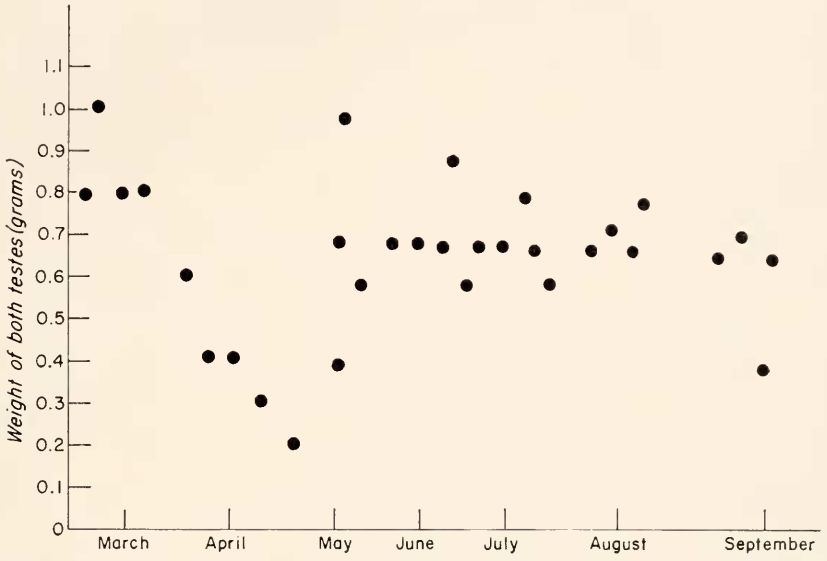

FIGURE 2. Seasonal fluctuations in testes weight in thirty specimens of *Chrysemys picta*.

Females were considered sexually mature if they: 1) contained follicles with diameters greater than 15 mm; 2) contained oviducal eggs; 3) were found mating; or 4) were found nesting. Only 7 females of 16 examined with plastron lengths below 100 mm contained follicles of 15 mm. The smallest had a plastron of 80.8 mm. Four had plastron lengths between 90 and 99 mm. All 7 showed 5 years growth annuli. All females examined over 100 mm plastron length contained some follicles of 15 mm. All of the females found containing oviducal eggs, mating, or nesting, were over 110 mm in plastron length and at least 5 years old. White Oak females were mature at 110 mm plastron length after their fifth year. Possibly some mature at a smaller size (between 100 and 110 mm). Cagle (1954) reported 20 Illinois females containing eggs were 126 to 160 m min plastron length, 14 Tennessee females 106 to 138 mm, and a single Michigan female was 152 mm. He assumed that a female having ovarian follicles 10 mm or greater in diameter was capable of depositing eggs; and found 41 Illinois females, plastron lengths

122 to 162 mm, and a single Louisiana female, length 125 mm, with follicles of this size. He stated that females became sexually mature when they reach a plastron length of 120 to 130 mm. Gibbons (1968) reported that sexual maturity in southwestern Michigan was attained when females reached a plastron length of about 110 to 120 mm (at about 10 years of age).

Male sexual cycle and maturity

Testes weighed the most during March following emergence from hibernation (Fig. 2). The testes contained much sperm at this time and additional weight was possibly due to the proliferation of the sustentacular cytoplasm. Weight decreased steadily during April and May as the sperm passed out of the testes, and into the epididymides. Weight increased in June as sperm maturation again began. The greatest diameter varied little, ranging from 6 to 8 mm during each month. Gibbons (1968) reported changes in the size of the testes. He reported the testes to be reduced in size from March to June, enlarged from July to September and to be small again in October. The changes in weight agree with those reported by Gibbons (1968) for Michigan *C. picta.*

Spermatogenesis began in March when a few spermatogonia first appeared. At this time there were many Sertoli cells present and some cellular detritus was contained in the lumen of the seminiferous tubules. Sperm produced in the previous cycle were also present in the lumen. As March progressed the clear cytoplasm of the cells extended into the lumen of the turtle.

During April there was much detritus in the lumen, and the sperm started to pass out of the tubules and into the epididymides. The cells bordering the tubule were no longer clear or extending into it. There were about an equal number of Sertoli cells as primary spermatocytes. As the month passed, a second layer of spermatocytes was formed.

In May there were two or three rows of spermatocytes, both primary and secondary, surrounding the Sertoli cells which were decreasing in number. Spermatids were now present in small numbers. Detritus and some sperm still remained in the lumen.

A few new sperm appeared around the border of the lumen in early June. Many of the lumens were now clear of detritus. Spermatids increased in numbers. Spermatocytes were present, but the Sertoli cells had practically disappeared. In late June many newly formed clumps of sperm could be seen around the lumen border.

July and August were the most active periods of sperm production. By late July large numbers of sperm were found clumped in the lumens and by the end of August filled most of the lumen and lined the borders.

During September the sperm moved to the center of the lumen so that few remained at the border at the end of the month. The cycle appeared complete by this time. Gibbons (1968) reported that the sperm passed into the epididymides during October. Figure 3 shows stages of the cycle.

Risley (1938) found the testes of *Sternotherus odoratus* to be largest in August and smallest in early May. Recession of testes in spring coincided with the mating period and later increases in size with increasing spermatogenic activity

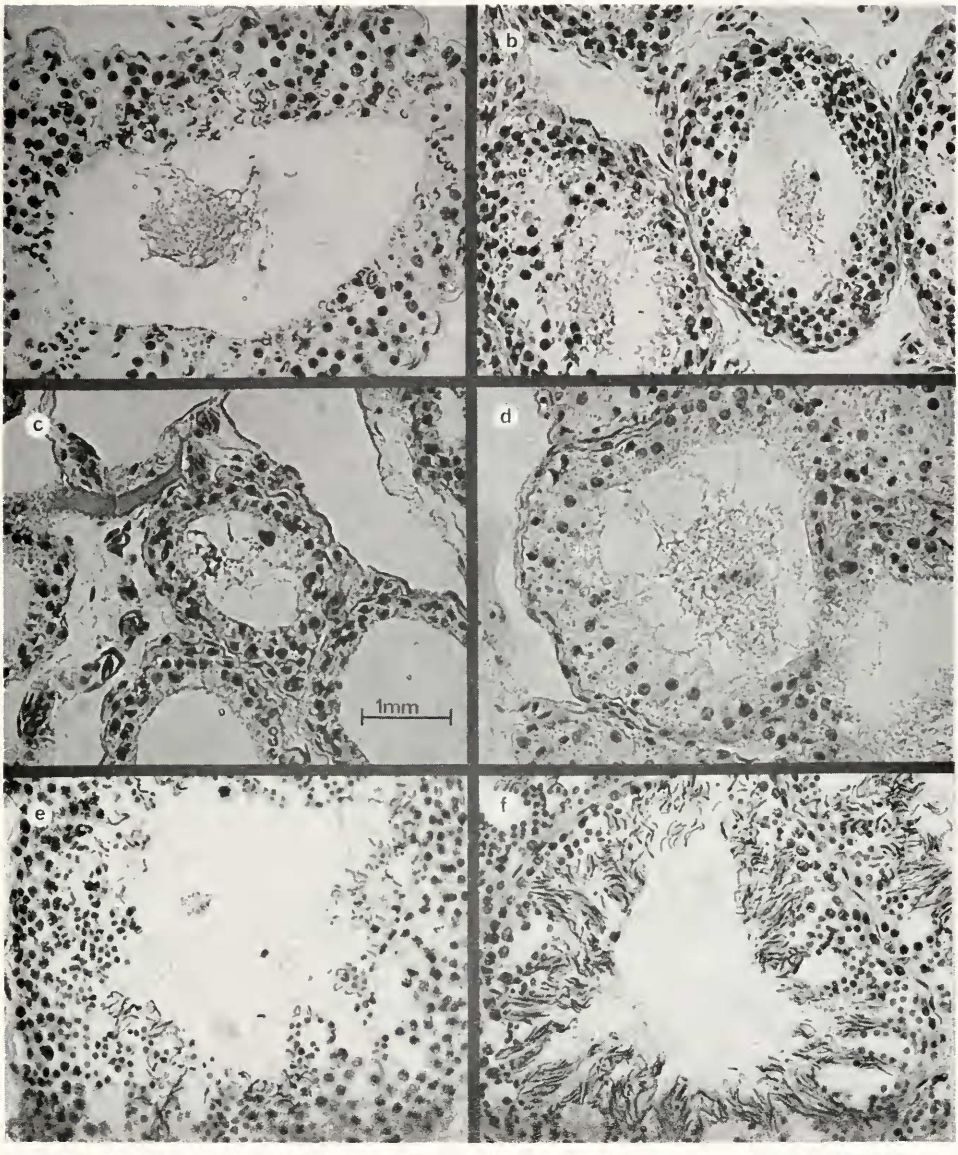

FIGURE 3. Representative stages in the spermatogenic cycle of *Chrysemys picta*. Letters a to f, respectively, are sections of testes obtained on 14 March, 4 April, 11 May, 12 June, 12 July, and 23 August.

and enlargement of the seminiferous tubules. Atland (1951) reported that the cycle of *Terrapene carolina* was essentially like that of *S. odoratus*. Legler (1960) showed the cycle of *Terrapene ornata* began in early May and ended in late October and except for a longer length was the same as that of *S. odoratus* and *T. carolina*. The spermatogenic cycle of *C. picta* beginning in March and ending in September

is even longer, but in main points does not differ from those species previously mentioned.

Male specimens of *C. picta* were considered sexually mature if they contained mature sperm in their testes or epididymides. The smallest turtle containing mature sperm had a plastron length of 87.8 mm and showed 5 growth annuli. This turtle was sacrificed on 19 September, 1966, and possibly had mated the previous spring. Mature males have elongated forefoot claws (over twice as long as those of the hind feet), and elongated preanal tail lengths (Table I). Males first showed these characters at 70 mm plastron length in their fourth year. Apparently, males mature during their fourth year in Pennsylvania, but do not mate until the spring of their fifth year. Gibbons (1968) reported that male *C. picta* in southwestern Michigan mature when they reach 80 mm plastron length and showed some to be mature in their fourth year. Cagle (1954) reported male *C. picta* reached maturity at 90 mm in Michigan, 70 mm in Illinois, and reported Louisiana males sexually mature at 55 and 62 mm, but other males in the size range 50–60 mm were sexually inactive. Cagle (1954) stated that males of the southern populations apparently may become sexually mature during the first year of life (in one complete growing season), and the northern males require at least two and possibly three seasons to attain maturity. Cagle (1948) reported male *Chrysemys scripta* normally become mature at plastron lengths of 90 to 100 mm but occasionally individuals may become mature at a smaller size. *Chrysemys scripta* in their first mature season may be 2 to 5 years old. The attainment of maturity in the genus *Chrysemys* is apparently a factor of size rather than age.

Discussion

The variations in the sexual cycles and attainment of maturity between the specimens of *Chrysemys picta* in this study and those studied by Cagle (1954), Powell (1967), and Gibbons (1968) are of interest.

Climatic conditions greatly influence the physiological activity of turtles. Cold temperatures reduce the metabolic rate of *Chrysemys picta* (Rapatz and Musacchia, 1957) and probably also reduce the activity of the reproductive organs. White Oak painted turtles were active in all months except February (Ernst, 1969), and although Gibbons' (1968) females had similar ovulatory periods to those from White Oak, the annual activity period in southwestern Michigan was shorter (Gibbons, 1967). Sexton (1959) also reported a shorter annual activity period during his study of *C. picta* in southern Michigan. Although no figures are available on the length of the annual activity period in Nova Scotia, it is probably also shorter than that in southeastern Pennsylvania. This is caused by the earlier onset of colder water temperatures and the delayed thawing of the ice cover in spring.

According to Goode (1953) both southern Michigan and Nova Scotia have a continental forest climate with cool summers while southeastern Pennsylvania has a continental forest climate with warm summers. Nova Scotia has a surface temperature below 32° F in winter and from 50° to 68° F in summer. Both southern Michigan and southeastern Pennsylvania have surface temperatures below 32° F in winter and above 68° F in summer. During January the normal temperatures of both southern Michigan and Nova Scotia range between 20° and 30° F, while

those of southeastern Pennsylvania range between 30° and 40° F. The normal July temperatures of southern Michigan and southeastern Pennsylvania range between 70° and 90° F while those of Nova Scotia only range between 50° and 70° F.

A shorter period of development caused by prolonged colder water temperatures could explain why the ovocytes of females from the more northern populations do not grow as large as do those from White Oak females. It would be interesting to compare data from Louisiana females. This could also explain the earlier maturity of both sexes at White Oak. Cagle (1954) has reported an inverse relationship between the attainment of sexual maturity and latitude in *Chrysemys picta,* as shown in the present study. Tinkle (1961) reported that in *Sternotherus odoratus* both southern males and females reach sexual maturity at a smaller size than do the northern sexes.

Inhibition by cooler temperatures may explain the lack of growth in ovocytes in Nova Scotia during the summer, but it is not clear why such a difference should exist between the Michigan and Pennsylvania populations which both have warm summers.

Painted turtles from more southern localities are known to have longer reproductive periods. Cagle (1954) defined the nesting season of *Chrysemys picta* as that period in which females may contain oviducal eggs and reported a nesting season of early April to the last of July in Louisiana and from 12 May to 22 July in Illinois. Presumably this difference is caused by the warmer climate in Louisiana.

Multiple nestings as supposed by Cagle (1954), Powell (1967), and Gibbons (1968) have never been proven. Since no female *Chrysemys picta* has been observed nesting more than once in a given season, this difference may not exist. If multiple nestings do occur they would be restricted to southern populations because of the short northern egg-laying season.

The difference in the sexual cycles and attainment of maturity shown in this study point out that too often the results of a study of one population of a species are fallaciously taken for granted as being true for all such populations throughout the species' range. Gibbons and Tinkle (1969) have pointed out similar variations in the clutch size of *C. picta* populations in a single geographic area. There is a critical need for more information on the factors affecting reproduction in turtle populations.

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Summary

1. A mature male *Chrysemys picta* can be distinguished from a female by its long forefoot claws, thickened base of the tail, and short post-anal tail length.

2. Males become sexually mature when they reach a plastron length of 80 to 90 mm. They usually mature in their fourth year, but probably do not mate until the spring of the fifth year.

3. All sexually mature females contain follicles with diameters greater than 15 mm. All females containing oviducal eggs found mating or nesting were over 110 mm in plastron length and at least five years old.

4. Sexual maturity is apparently a factor of size rather than age.

5. Spermatogenesis begins in March with the most active period of sperm production occurring in July and August. The cycle is completed in September when the sperm start to pass into the epididymis where they are stored during the winter.

6. The ovarian cycle in southeastern Pennsylvania begins in July and August after ovulation and nesting, and is completed by the end of October. No change occurs during the winter. This differs slightly from the cycle of more northern females.

7. The ovarian activity alternates, and extrauterine migration of ova is known to occur.

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